A NOVEL METHOD TO QUANTIFY NUMBER OF OSTEOCYTE PROCESSES IN BONE

Michele Casanova[1,2], Aaron Schindeler[2], David Little[2], Philipp Schneider[1], Ralph Müller[1]
[1] *Institute for Biomechanics, ETH Zurich, Zurich, Switzerland*
[2] *Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Westmead, Australia*

Introduction

Osteocytes and their cell processes are key for bone mechanosensation [Jacobs, 2010] and they have been identified to act as mechanotransducers, inducing bone remodeling activities [Burra, 2011]. Hence, the number of cell processes is presumably an indicator of the bone's ability to respond to mechanical stimuli. Quantification of the osteocyte network has been often restricted to small regions encompassing a limited number of osteocytes [Schneider, 2010], without the possibility of a rapid quantification of the processes in a relevant volume including several osteocytes, which is essential for preclinical studies. Due to its relative low cost, fluorescent microscopy is commonly used for cell process visualization. Here, we propose an efficient fluorescent microscopy method to quantify cell process number and density.

Methods

A murine tibia was transversally cryosectioned to obtain 12 µm thick slices within the mid-diaphysis. The sample was dehydrated, left for 3 hours in 1% fluorescein isothiocyanate (FITC), and rinsed. The specimens were then visualized under a deconvolution microscope (DM) (DeltaVision). Seven regions in the middle of the cortex were selected ($103x103\mu m^2$) for further analysis. Each region of interest or ROI (ellipsoid in Fig. 1, n=21) encompassed one osteocyte. Since osteocyte processes fan out radially, the upper limit of the imaged ROI was placed about 1 µm above the cell body (Fig. 1B) to ensure that the processes pass the ROI at the smallest possible angle. For each region, 60 optical sections at 0.2 µm intervals were acquired between the cell body (Fig. 1A) and the upper limit (Fig. 1B) to sample the ROI. The images were analyzed using ImageJ (U.S. National Institutes of Health), including edge enhancement and absolute thresholding. The manually counted number of processes (Fig. 1C) was then divided by the ROI surface and volume to provide 2D cell process density.

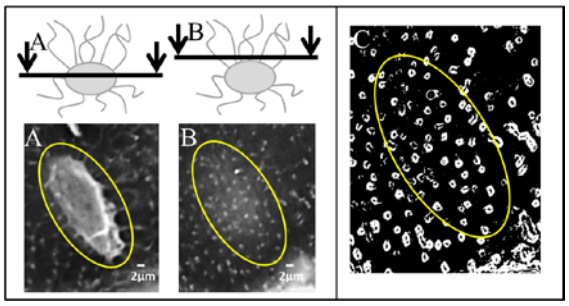

Figure 1: Representation of the method to quantify number of osteocyte processes. (A) The osteocyte is included in the ROI (B) A confocal plane of about 1µm above the osteocyte is selected. (C) The images were then processed for counting.

Results and Discussion

The calculated cell process density was 0.169 ± $0.028\mu m^{-2}$. The proposed method to quantify the number of osteocyte processes was found to be consistent for different regions hundreds of micrometers apart (coefficient of variation ±17%). In addition, it is reliable and fast, mainly because no 3D reconstruction of the osteocyte network is required and because images were recorded by deconvolution microscopy, offering strong signals from weak stains [Wallace, 2001], which was found to yield better contrast than our confocal microscope (state-of-the-art system).

Outlook

Since the osteocyte network distribution seems to be anisotropic [Beno, 2006] the proposed method has the potential to study number density changes in other spatial planes as well. Moreover, the time efficiency of the method makes it an ideal tool to compare inter-sample difference in cell process density in large studies.

References

Jacobs CR *et al*, Annu Rev Biomed Eng, 12:369-400, 2010.
Beno *et al*, J Biomech, 39:2378-87, 2006.
Burra S *et al*, PNAS, 107:13648-53, 2010.
Schneider P *et al*, Bone, 47:848-58, 2010.
Wallace W *et al*, Biotech, 31:1076-1097, 2001.